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FURTHER EXPERIENCES WITH BRILLIANT GREEN AGAR AND OTHER PLATING MEDIUMS FOR THE ISOLATION OF TYPHOID AND PARATYPHOID BACILLI FROM FECES

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Since describing a method of preparing a brilliant green agar plating medium for the isolation of typhoid and paratyphoid bacilli from feces,[1] the number of specimens examined has reached a total of over 7,000. It seems advisable therefore to present the results obtained as well as slight modifications of the methods employed as dictated by the experience gained in handling this large material. Since our report other observers have suggested various plating mediums for fecal examinations, and these have been tried sufficiently to determine their practical value. Our opinion as to their merits, as based on our experience, is also given.

As we have modified the preparation and standardization of our medium somewhat and to avoid the inconvenience of reference to our former report, it seems advisable to describe the medium and the methods as now employed.

Preparation of Medium.—The composition of the agar base is as follows:

Liebig's Extract of Beef	3 gm.
Peptone	10 gm.
NaCl	5 gm.
Agar	15 gm.
Water	1 liter

The agar is dissolved in half the water by autoclave, the other ingredients in the remainder of the water by heating on a gas stove. After complete solution the two halves are mixed and the reaction adjusted by the addition of alkali. The final reaction desired is the neutral point of the Andrade indicator. This indicator is prepared by adding 16 c c of N/1 NaOH solution to 100 c c of a 0.5% aqueous solution of acid fuchsin. The alkali changes the red of the fuchsin to orange or yellow. Mediums adjusted to the neutral

Received for publication April 8, 1918.

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[1] Krumwiede, C., Jr., Pratt, J. S., and McWilliams, H. I.: Jour. Infect. Dis., 1916, 18, p. 1.

point of this indicator are pink when hot and colorless when cold, which corresponds to between 0.6% and 0.7% acid to phenolphthalein (hot titration). The reaction of the agar may be adjusted to the phenolphthalein reaction given or directly adjusted to the Andrade indicator by adding 1% of the indicator to a small measured volume and determining how much alkali is needed to bring it to the point desired, namely, pink when hot and colorless when cold. After adding the appropriate amount of alkali, thus determined, to the bulk of the medium, the latter should be retested and readjusted if necessary. The Andrade indicator adjusts itself somewhat slowly to changes in reaction; a little time therefore should be allowed to elapse between each addition of soda. We have found that with Fairchild's peptone and Liebig's extract that the addition of 8 c c of normal soda per liter will give a satisfactory end reaction. This will vary depending with the brand of peptone and extract employed, but once the necessary amount of soda is determined, the reaction can be closely approximated by employing this amount in succeeding batches of medium, avoiding in this way the necessity of titration. After adjusting the reaction, the agar medium is cooled, beaten egg white added and then boiled and filtered. After filtration the entire preparation is mixed thoroughly to insure uniformity of the whole product, bottled 100 c c to a bottle and autoclaved for 30 minutes at 15 lbs. pressure.

At the time of use the needed number of bottles are melted and to each 100 c c of agar is added 1 c c of the Andrade indicator, 1% of lactose, 0.1% of glucose and finally the appropriate amount of brilliant green. For the addition of the carbohydrates it is most convenient to prepare a stock solution in distilled water, containing 20% of the lactose and 2% of glucose, sterilizing the solution by steaming in the Arnold sterilizer. Five c c of this solution added to 100 c c of agar will give approximately the percentages required. The brilliant green is added from a stock 0.1% solution in distilled water. Two concentrations of brilliant green are employed, the appropriate amounts being determined by preliminary standardization (see below under standardization). The medium is mixed, poured into plates so as to give a rather thick layer allowing 6 plates for every 100 c c of medium. The finished plates must be dry and this is best assured by using porous earthenware covers. With these, the medium can be covered as quickly as poured.

Standardization.—This is the difficult step and if not successfully carried out, much or all of the value of the medium is lost. In our previous report we advised the use of the Rawlings strain of B. typhosus for standardization. This strain, however, has fluctuated so much in its susceptibility to brilliant green that its use has been discontinued. Freshly isolated cultures have given somewhat better results but positive stools, if obtainable, are by far the more reliable material for this purpose.

The stock brilliant green solution will keep for about a month. A fresh solution therefore should be employed in standardizing a new batch of agar and the batch of medium should be as large as possible so that the number of standardization tests will be reduced to a minimum.

As each new batch of medium is received plates are poured using 3 dilutions of the dye, namely, 1:200,000; 1:330,000, and 1:500,000. For convenience these are expressed in terms of the fractions of a c c of a 0.1% solution of brilliant green added to a 100 c c of agar; the amounts are, respectively, 0.5, 0.3, and 0.2 c c. The plates are then inoculated with equal amounts of material, spreading it by means of a spatulum. A nonrestraining medium such as Endo is used as a control. The best material for standardization are positive stools from typhoid carriers or cases. If obtainable, several fecal specimens should be inoculated to compensate for the variability of the fecal flora, slight variations in resistance of different strains of B. typhosus, and to average the variables due to technic. If only one positive stool is available this should be plated in duplicate or used to inoculate suspensions of 2 or 3 normal stools which are then used for inoculation. The suspensions should be comparatively light so that the resulting plates will have discrete colonies, otherwise comparison by count or estimate will be difficult or impossible.

The stools should be freshly collected as the persistent types in older stools tend to be the dye-resistant types and are therefore not satisfactory as a measure of the restraining action of the dye on the average fecal flora. If positive stools are not available, suspensions of fresh normal stools may be inoculated with broth cultures of recently isolated typhoid strains and the plates inoculated. The addition of a loop to 0.1 c c of an 18-hour broth culture to 10 c c of stool suspension of moderate density will give a well balanced mixture of B. typhosus and fecal bacteria. Pure cultures of B. coli should not be used to determine the restraining action of the dye. The degree of restraint of the average fecal flora is the fact wanted.

After incubation the plates are inspected and based on the results, selections made of the dilutions to be used. Two dilutions are chosen: first, the greatest amount of dye which has little effect on the number and size of the typhoid colonies, but which shows a marked restraint of the fecal flora; second, a greater amount which shows a moderate reduction in the number and size of the typhoid colonies and usually a still greater reduction of the fecal flora. Slightly greater concentrations of dye are used in practice than would seem to be indicated by the standardization results as the inoculation will be heavier, thus reducing the activity of the dye to some extent. With most batches of mediums and with the sample of dye we employ the 2 optimum dilutions average 0.2-0.3 c c, respectively, of a 0.1% solution of the dye to 100 c c of agar. (See brands of dye.) The reasons and advantages of two concentrations of the dye are given later.

Interpretation of the results of standardization tests and the selection of the optimum dilutions are most easily and accurately determined by a graphic representation of the results. Averages are taken of the figures recorded for the different specimens on the Endo medium and on the dye agar plates according to concentrations taken and the following data charted: the average percentage reduction on the dye plates of (a) colonies of fecal bacteria other than typhoid, (b) typhoid colonies, and (c) reduction in size of typhoid colonies using 1 mm. as the standard. In the accompanying representative charts (Charts 1, 2, 3, and 4) the results with fecal typhoid and culture typhoid strains are given separately for comparison. The selections that should be made for routine use are indicated by a heavy vertical arrow. The curves show clearly the basis of selection. Charts 1 and 2 are representative of the general limits of fluctuation with different batches of agar with the dye we have used in our routine work.

The two dilutions selected according to this method serve well in routine work for the isolation of both B. typhosus and B. paratyphosus. When the latter is the only type to be sought, one strong concentration may be employed alone, as this type is much more resistant to the dye than B. typhosus. An advantage of using stronger concentrations in this case would be the greater degree of restraint of the accompanying fecal flora. With a standardization result indicating that 0.2 c c and 0.3 c c are satisfactory for the isolation of B. typhosus from 0.35-0.5 c c can be used for B. paratyphosus. During an outbreak of paratyphoid fever, where one strain is presumably common to all the cases and carriers, the medium can be directly standardized, using a positive stool or a freshly isolated strain as soon as one is obtained. Because of the greater resistance of B. paratyphosus, wider limits of error are allowable than in standardizing for B. typhosus, and freshly isolated cultures give very satisfactory results. We have recently employed this method with marked success in the case of a regiment infected with B. paratyphosus A.[2]

Adaptability of Various Brands of Dyes.—Various samples of dyes have been tried. Of these the Bayer, Grübler and Hoechst brands have given successful results and the dilution range of differential action was closely alike. Of the other samples, bearing no name but probably of German manufacture, some have been fairly good. Examples are given in Charts 3 and 4. Although good differential action was obtained with both samples a considerable concentration was needed, indicating that there were inactive impurities present. Although we have not personally encountered dye samples which, even when very dilute, inhibited B. typhosus as well as the fecal flora, we have had a report of such an experience. The results would seem to indicate the presence of highly toxic impurity.

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[2] Krumwiede, C., Jr.: Jour. Infect. Dis., 1917, 21, p. 141.

Colony Characteristics.—On the medium, after 18 hours' incubation, the typhoid colonies will be found to be very characteristic. They are of good size (1-1.5 mm.); the glucose not only enhances their growth but is also the main cause of their characteristic appearance. Viewed through the plate against a dark background, the light passing obliquely through the agar, they have a peculiar striated, flaky appearance. With artificial light and a hand lens, under the same conditions

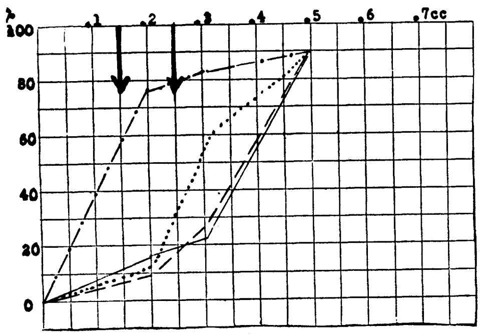

Chart 1.

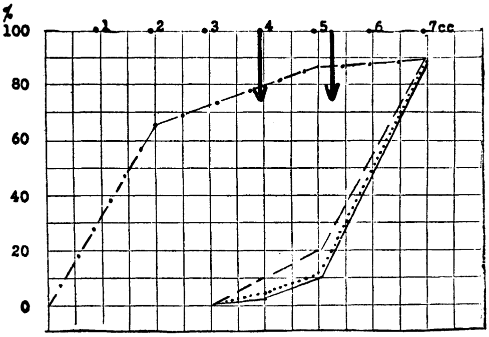

Chart 3.

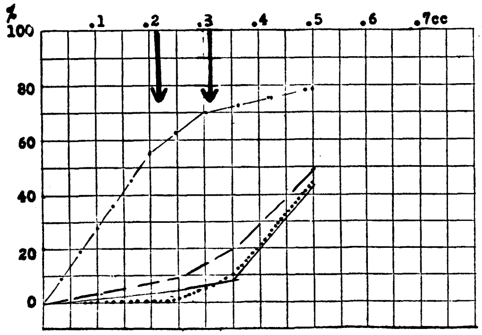

Chart 2.

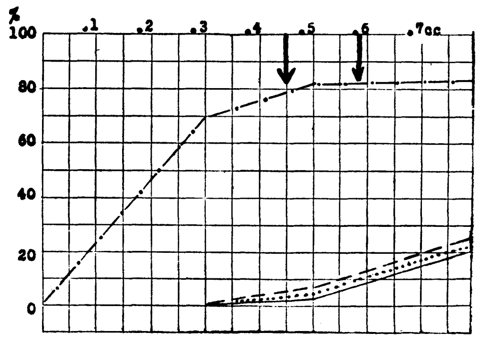

Chart 4.

Explanation of charts: —.—.—.— = Fecal types; ———————— = Fecal typhoid; .... = Culture typhoid; — — — — — — Size of typhoid colonies.
Charts 1 and 2.—Standardization test. Bayer dye.
Charts 3 and 4.—Standardization test. Dye? See under Brands of Dye.

they have the appearance of a coarse wool fabric. They may take on a faint mauve tinge from fermentation of the trace of glucose, which accentuates somewhat their characteristic appearance. The larger colonies may resemble the paratyphoid B type. Colonies of this organism tend to be larger, heavier and more opaque. They are often tinted a delicate green, and the markings are less distinct, though still evident at the periphery. The paratyphoid A colonies resemble the typhoid colony more nearly than that of the paratyphoid B, but we have frequently observed an extremely flattened, slightly tinted colony whose edges melt into the agar. The markings of the colonies of all three organisms are usually more prominent on the stronger dye plates. The only organisms which simulate the typhoid colony are certain partially restrained colon types which may develop on weak dye plates. They present exaggerated markings, with distinct cross-bar striations, and they are usually completely excluded on the stronger dye plates. These organisms frequently agglutinate spontaneously in the saline drop (see below). Some of the dye-resistant "intermediates" develop paratyphoid-like colonies.

With no known change in the mode of preparation an occasional batch of agar will develop very large typhoid colonies. These luxuriant colonies tend to be much less characteristic in their markings, and the tendency to the development of this type should be noted when standardization tests are made, so that such colonies will not be overlooked in using the medium subsequently in routine work. With an agar of this kind slight inhibition by the dye will result in more typical colonies apparently due to a lessening of the density of the colony, thus making the markings more visible. It is possible even with the average batch of agar that the dye, exerting a very slight influence, contributes somewhat to the development of the typical colony.

The colonies of the fecal types which develop take on a moderate pink color if they ferment lactose, but the color change is not marked. Even without a sharp color change, they are so different from the typhoid or paratyphoid colony that the practiced eye passes over them without hesitation. This lack of sharp color change may seem a decided disadvantage to bacteriologists accustomed to the sharp color differences of Endo and similar mediums, on which the typhoid-paratyphoid colonies are presumably those showing no change of indicator. We have approached the problem, however, from the standpoint of giving the colonies of these types positive characteristics, rather than relying on a negative characteristic, inability to ferment lactose. In this regard, the medium is unique, and, to our mind, the more logical way of attacking the problem of evolving a still more satisfactory medium.

Method of Inoculation.—To obtain discrete colonies various methods of inoculation are advised, as for instance the use of successive dilutions of an initial fecal suspension. Such a method takes time and glassware and is not very expedient in handling large numbers of specimens. With some practice one can obtain very satisfactory results by diluting the suspension to a density which experience has shown will yield sufficiently abundant but discrete colonies. This density corresponds roughly to a suspension of 1 part of solid feces to 15 parts of fluid. We dilute fluid feces or emulsify solid feces to this density using peptone water and allow the suspensions to stand 15-30 minutes to allow sedimentation of the particles and then inoculate the plates from the surface of the suspension. To save time inoculation is done with a double loop so that one or both may be emptied on the plate. For each specimen 2 series of plates are employed consisting of a weak and a strong dye agar and Endo plate. The fecal suspension is inoculated only on the dye plates, 1 loop being placed on each of the first series and 2 loops on each of the other series. The material is then spread with a bent nichrome wire, rubbing out the material on the weak plate, then on the strong plate and then passing to the Endo plate, the material adhering to the wire sufficing to inoculate this plate. The second series is then streaked in the same fashion. The stronger dye-plate in each series receives in this way the heaviest inoculation. The spreader is only sterilized between specimens.

Nichrome wire is a cheap and convenient substitute for platinum and a very satisfactory spreader is made by bending the wire to an angle of 120° and bending up the tip to avoid tearing the plates. It does not fuse well in glass and the shank is best bent in a zigzag fashion and pushed into narrow bore tubing with thick wall. Owing to its greater rigidity a finer gage of nichrome wire can be used than in the case of platinum, thus compensating for the slower cooling of the former. If 2 spreaders are used, 1 cools while the other is in use. We have found the wire spreader more convenient than glass rods which cool very slowly if sterilized by heat while inoculating, and are inconvenient to wash and sterilize in bundles especially when the number needed is large.

Identification.—Although the preceding gives the details of the medium it seems well to describe again the following methods because of their expediency and reliability in mass work. Preliminary identification of the colonies is done by means of the slide agglutination method using highly potent serum. A drop of highly immune serum in low dilution is placed on a slide, and beside it one of salt solution. The suspicious colony is picked off with a small loop of fine wire, and a sufficient amount rubbed off in the salt solution to give a slight clouding. Some of the growth remaining on the loop is then rubbed off similarly in the serum drop. An immediate clumping, visible to the naked eye is a positive result; spontaneous agglutination will be noted in the control. When the colony is suspicious of paratyphoid, 3 drops, salt solution, A and B serum, may be used, a portion of the colony being rubbed in the salt-solution and one serum, and another portion in the second serum, with intermediate sterilization of the loop. It is essential, for this purpose, that the serum be of high titer, as a slow reaction is time-consuming and permits drying of the drop. In general, we prefer an older serum of original high potency, which has lost not only in total titer for its homologous type, but also in content of group and normal agglutinins. Under these conditions, the dilutions used may be as low as 1 : 50 to 1 : 100. With a known and tested serum, the results are very reliable; with an unknown serum, serious error may result because of group reaction. Occasionally a slight delay in clumping, due to the prezone phenomenon, will be noticed, but there is usually some indication of a reaction in the serum, and dilution with an additional loop or two of salt solution induces prompt and marked clumping. We have noted increased agglutinability in colonies on dye plates as compared with those on Endo. This is apparently due not to the dye alone but to the glucose as well; in any case, it is no disadvantage. Inagglutinable strains, showing no reaction in the concentrations of serum employed, are rarely isolated from feces, but a characteristic colony, nonagglutinating, should not be overlooked. In emergencies, with known serums and trained workers, the macroscopic slide agglutination could be considered final for all promptly positive cases with little likelihood of error. The doubtful slower reactions, usually few in number, would have to be verified by isolation. A negative slide agglutination is usually accepted as a final negative result, but we fish some suspicious colonies occasionally as a control on the serum. Positive results are checked by fishing to the Russell medium or better a modified Russell containing 1% of saccharose as well as 1% of lactose and 0.1% of glucose.[3] Paratyphoid-like types which may give some reaction with the paratyphoid serums are more surely excluded in this way, as many of these types ferment saccharose but attack lactose only slightly or not at all. If the reaction on the Russell medium is typical the results of the slide agglutination are verified by using the growth for macroscopic agglutination. If the serums used are of sufficient titer and the range of group and normal agglutinins is known, this suffices for all practical purposes. If the serums are not altogether satisfactory false positive results are likely to be encountered in examining for B. paratyphosus and the results are then best verified by determination of the cultural and fermentative characteristics.

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[3] Krumwiede, C., Jr., and Kohn, L. A.: Jour. Med. Research, 1917, 37, p. 225.

If the search is to include the paratyphoid types causing food poisoning (B. cholerae suis and B. enteritidis) fishing of the suspicious colonies becomes necessary as well as the full determination of the cultural and fermentative characteristics and final identification by agglutination with the appropriate serums and possibly agglutinin absorption.

There is one source of error in the use of brilliant green agar which is common to all selective mediums. In fishing a suspected colony, impure cultures may be obtained, as fecal bacteria which have been inhibited are capable of multiplication when transferred to a favorable medium. This danger should be kept in mind, and suitable precautions taken. In fishing, it is well to avoid touching anything but the apex of the colony. It is well to restreak on an Endo plate if there are very few positive colonies in a crowded area and it is questionable whether a pure culture can be obtained.

Results Obtained with the Brilliant Green Medium.—Table 1 gives the numerical results in the isolation of B. typhosus from feces.

TABLE 1

FECAL EXAMINATION FOR B. TYPHOSUS

Source	Totals	Endo Positive	Dye-Agar Positive	Percentage of Endo Positives Compared with Total Positives
All sources..................	7,111	920	1,235	74.5
From carriers..............	594	502	564	89
All sources exclusive of carriers.................	6,517	418	671	62

As one would expect, the chronic carriers give the least increase in positive results as their feces usually contain a relative abundance of typhoid bacilli allowing of easy isolation with any nonrestraining medium. The fewer the bacilli in proportion to other fecal types the greater should be the expected increase of positive results with the dye-medium. This expectation is realized and shown in the results in Table 2.

TABLE 2

SPECIMENS FOR DIAGNOSIS FROM SUSPECTED CASES OF TYPHOID FEVER

Number	Endo Positive	Dye-Agar Positive	Percentage of Endo Positives Compared with Total Positives
115	18	45	40

Table 3 is of interest in this connection. The examinations were carried out in making a sanitary survey of an insane asylum because of the development of cases of typhoid fever. Nearly all of the positive results were obtained from patients found to be suffering from the disease. Because of their mental state the existence of the disease was unsuspected in some of the patients till positive fecal reports led to routine temperature taking. A rise of temperature was frequently the only indication of the presence of the disease.

TABLE 3
SPECIMENS, STATE INSANE ASYLUM

Number	Endo Positive	Dye-Agar Positive	Percentage of Endo Positives Compared with Total Positives
847	30	46	65

Although the figures indicate the numerical increase of positive results on the dye-agar they do not indicate the greater ease of isolation on this medium in cases in which the Endo medium is positive as well. With a dye-sensitive fecal flora as encountered in the average feces, the suppression of these types on the more heavily inoculated dye-plates results in the development of many more typhoid colonies as compared with the Endo plates or may result in the development of typhoid colonies alone. The contrast between the Endo and dye-plates in the actual number of typhoid colonies and the comparative number in relation to the total number of colonies is usually very striking.

We have continued the use of the Endo medium mainly to obtain comparative data and to some extent as a check on the use of the brilliant green medium because of a frequent change of personnel among those carrying out the actual examinations. With sufficient experience it could be omitted as we have encountered positive results on Endo with negative results on the brilliant green agar in only two instances. In one this seemed to be purely accidental; in the other the dye-plates were overgrown by B. aerogenes types, the feces being unusually rich in this dye resistant variety.

Two concentrations of dye are used for the following reasons: Brilliant green is an exceedingly sensitive colloidal restraining agent and its differential action is quantitative in nature, being elicited only at certain dilutions. The activity of the dye is not necessarily the same in different batches of medium apparently prepared in an identical manner. The restraining action is elicited in higher dilutions in a clear than in a cloudy agar, and any foreign matter, a trace of cotton in the agar, for example, will reduce the activity of the dye. For this reason the restraining action will vary with the amount of fecal protein inoculated. Furthermore, the fecal flora of different specimens varies somewhat in dye sensitiveness. Strains of B. typhosus likewise vary slightly in their resistance. Two dilutions compensate for these variables and for any slight miscalculation in the selection of the dilutions as based on the standardization test. The stronger dye-plate has one advantage that, although there be a reduction in the number of typhoid colonies developing, the greater restraint of the fecal flora may bring them into greater relief. Table 4 gives the comparative results with the 2 strengths of dye; it does not show, however, the greater preponderance of B. typhosus on one or the other concentration which is frequently encountered in examining the plates from an individual specimen. This must be considered in interpreting the results given, especially the greater number of positive results with the stronger concentration of dye.

TABLE 4

FECAL EXAMINATION FOR B. TYPHOSUS: COMPARATIVE RESULTS ACCORDING TO STRENGTH OF DYE

Positive on weak dye-agar only	26
Positive on strong dye-agar only	81
Positive on both weak and strong dye-agar	1,128
Total positive on dye-agar	1,235
Total positive on Endo	920

The results in the isolation of B. paratyphosus (Tables 5 and 6) have been somewhat better than in the isolation of B. typhosus.

On the whole the results obtained have equaled those originally reported as based on only a relatively small series of examinations.

In our original communication,[1] we recommended Kendall's modification of Endo's medium because of the convenience of using the same agar stock for both this and the dye-agar. We have found, however, that although Kendall's modification gives excellent differentiation for the more vigorous lactose fermenters, a great many colonies which were absolutely colorless on this medium, ferment lactose rapidly when transferred to Russell's double sugar medium. We have tried numerous other modifications of Endo and have found the one recently recommended by Robinson and Rettger[4] to be the most satisfactory, giving as it does the sharpest color to the lactose fermenting colonies with the least diffusion. We have changed their method slightly to avoid adding the indicator till the medium is to be used, as there is always the danger of the reduced color returning, especially if large batches of mediums are prepared at one time.

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[4] Jour. Med. Research, 1916, 34, p. 363.

The medium is prepared as follows: Twenty-five gm. of agar, 10 gm. of peptone, and 5 gm. of meat extract to a liter of water, are dissolved by heating on a gas stove. The ingredients are made neutral to litmus by the addition of 10% solution of sodium carbonate. A further 10 c c of sodium carbonate is added per liter. The medium is cooled to 45 C. and one egg per liter added. Autoclave for 30 minutes at 15 lbs. pressure. Filter and add 1% lactose and bring to a boil to dissolve the sugar. Finally add another 10 c c of 10% solution of sodium carbonate per liter, the reaction should now be 0.3 — to phenolphthalein (hot titration). The medium is bottled in 100 c c amounts and autoclaved for 10 minutes at 10 lbs. pressure. To the melted agar just before use, add 0.5 c c of a saturated alcoholic solution of fuchsin and 1 c c of a 10% solution of amorphous sodium bisulphate.

TABLE 5

FECAL EXAMINATIONS FOR B. PARATYPHOSUS A

Number	Endo Positive	Dye-Agar Positive*	Percentage of Endo Positives Compared with Total Positives
856	30	60	50

* Majority of results based on use of one strength, 0.35-0.5 c c of a 0.1% solution of dye to 100 c c of agar.

TABLE 6

B. PARATYPHOSUS B

Number	Endo Positive	Dye-Agar Positive	Percentage of Endo Positives Compared with Total Positives
(11)*	5	11	45

* Encountered while examining for B. typhosus.

If, due to heavy seeding, diffusion occurs, typhoid colonies may nevertheless be identified on this medium by the characteristic translucent quality of the typhoid colony, which distinguishes it from the metallic sheen exhibited in most instances by even the smallest colony of the colon types. In case this distinctive appearance of the typhoid colony is not clearly perceived by daylight, this quality may be easily brought out by artificial illumination.

While this work was in progress, two plating mediums were reported: an eosin-methylene blue medium by Holt-Harris and Teague,[5] and an eosin-brilliant green medium by Teague and Clurman.[6] The eosin-methylene blue medium depends on the application of the protective colloid principle. The 2 dye-solutions which in the concentrations used would mutually precipitate in aqueous solution, are prevented from combining when added separately to the agar, due to the protective action of the colloids in the medium. When fermentation occurs in the development of a colony, the protective action of the colloids is destroyed by the products of fermentation at this point, precipitation occurs, and the fermenting colony assumes a contrasting color.

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[5] Jour. Infect. Dis., 1916, 18, p. 596.

The eosin-methylene blue medium has no differential restraining action on B. coli, and was suggested as a substitute for Endo's medium. We have worked with this medium and agree with the observations of Holt-Harris and Teague as to the sharp differentiation of the fermenting and nonfermenting types, the absence of any diffusion even with heavy seeding, and the lack of sensitiveness to the exposure of light. Since no diffusion occurs, a 1½% agar may be used as the basis of the medium instead of the 2½% agar usually required for Endo, which is a distinct advantage. Holt-Harris and Teague note one drawback to the eosin-methylene blue medium: the tendency of the reaction to disappear when growth is crowded, but claim that the medium will give successful differentiation, when an Endo plate equally inoculated would be unworkable on account of the diffusion of the indicator. We are not ready to agree with this contention. With a satisfactory Endo medium even an apparently hopeless plate can be fished by the aid of artificial illumination, the translucent, glassy typhoid colony being easily differentiated from the opaque, more deeply colored fermenting colonies which, even though small, nearly always show a distinctive metallic sheen. With diffusion on Endo, unless it is very intense, the search for the translucent typhoid colony seems to us no more difficult than on the equally deeply colored eosin-methylene blue agar. The colored background to our mind is the essential objection to the medium. In examining a large number of plates, it strains and tires the eyes rapidly, and obscures the differences between the typhoid colonies and other nonfermenting types, which are usually perceptible on Endo, to those accustomed to its use. Both mediums have advantages, and the selection must depend on personal preferences. For occasional or small series of examinations, the eosin-methylene blue medium would probably be preferable because of the stability of the reagents used and the resulting greater uniformity of the product. We have found the extract agar we use for the brilliant green plating medium, to be a satisfactory basis for the eosin-methylene blue medium. Because of the transparent background and the tendency of the typhoid colony to take on positive characteristics, a properly standardized Endo plate gives the best picture obtainable, and because of these facts as well as the large number of examinations, we have continued its use in spite of its other drawbacks.

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[6] Jour. Infect. Dis., 1916, 18, p. 647.

We have also tried the eosin-methylene blue medium with members of the paratyphoid and dysentery groups. With B. paratyphosus A and B, the results were as satisfactory as with B. typhosus. The dysentery types, particularly the Shiga, are apt to be irregularly restrained on this medium. Since these observations are based on stock cultures, they are only to be interpreted as a caution, as we have had no experience with freshly isolated fecal strains. We have noticed a similar tendency to irregular restraint, with dysentery strains on Robinson and Rettger's modification of Endo. Kendall's Endo has not shown this action if the plates were freshly poured.

The color change on the eosin-brilliant green medium of Teague and Clurman is less sharp than with the eosin-methylene blue medium. The essential claim made for the eosin-brilliant green medium was that the eosin acting probably by absorption reduces the activity of the brilliant green to a base level where it is no longer appreciably influenced by differences in the medium or by the fecal proteins inoculated. We have tried this medium to only a limited extent because of the difficulties encountered in obtaining what we considered a satisfactory end-product. These difficulties were in relation to obtaining suitable differential restraint and were probably due to the fact that a sufficient range of dilutions were not tried. In a later article, Teague and Clurman[7] suggest the preliminary use of several concentrations of brilliant green and that large batches of agar be prepared to limit the number of preliminary tests of the medium. In other words, the masking action of the eosin does not exclude the necessity of preliminary standardization and the determination of the optimum concentration of brilliant green in the routine examinations. There are other and more essential disadvantages to the medium. A meat-infusion agar is necessary. The comparatively deep color of the medium obscures any positive characteristics the typhoid colony may possess, eye-strain resulting from the attempt to differentiate the nonlactose fermenting types. The eye-strain is enhanced by the peculiarly irritating color of the medium when viewed by transmitted light. This becomes a very serious drawback when the plates to be examined by one individual run into the hundreds. Both these difficulties are further enhanced when the plates become crowded as the differential color reaction tends to disappear. Since standardization is necessary, an almost colorless transparent medium which, however crowded, still retains the characteristic appearance of the typhoid colony seems to us preferable.

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[7] Jour. Med. Research, 1916, 35, p. 121.

SUMMARY

The brilliant green medium we have devised has markedly increased the number of positive fecal examinations for B. typhosus and B. paratyphosus. The methods of preliminary and final identification previously recommended have been found reliable and have materially aided in making possible large numbers of examinations with the minimum of workers. The essential point in the success of the medium is proper standardization. No method has been devised to render such standardization unnecessary. The medium is most adapted to routine work where many specimens are examined, as in this case the time required for standardization is relatively slight, as large amounts of medium can be standardized and used before the results of the standardization tests are rendered void by the deterioration of the dye solution or evaporation of the medium. Of the recently suggested nonrestraining plating mediums, Robinson and Rettger's modification of the Endo agar has given a far superior contrast between lactose and nonlactose fermentation types than that obtainable with the various modifications thus far tried. The methylene blue-eosin medium of Holt-Harris and Teague gives a similarly striking contrast if the plates are not crowded, but has the objection of being a colored medium thus obscuring any differeneces between the colonies of the nonlactose types and tiring the eyes when numerous plates are to be examined. Both of these mediums tend to restrain the growth of some culture strains of B. dysenteriae, indicating that they may not be satisfactory for the isolation of this organism from feces.